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13. ABSTRACT (Maximum 200 Words)

The goal of this project is to characterize T cell tolerance to prostate tumor antigens and identify the role of costimulatory receptors in overcoming this tolerance. Identification of these processes will assist in the development of novel therapeutic approaches for treating prostate cancer. We use the TRAMP model, a transgenic mouse line that develops primary prostatic tumors due to expression of the SV40 T antigen (TAg) under the transcriptional control of a prostate-specific promoter. In this summary, we report that adoptive transfer of naive TAg-specific T cells into TRAMP mice results in an initial proliferative expansion followed by a clonal deletion within 5 days. Vaccination of mice with dendritic cells pulsed with the cognate antigen results in expansion in the peripheral lymphoid compartments and subsequent trafficking to the prostate-draining lymph nodes (pDLN). However, 11 days post-vaccine, Ag-specific T cells were no longer detectable in pDLN. In contrast, a large percentage of Ag-specific T cells were still present in the prostatic tissue of vaccinated TRAMP mice, and persisted for at least 21 days. These data demonstrate that an Ag-pulsed DC vaccine may "rescue" prostate-specific T cells from peripheral tolerance in tumor-bearing mice and promote their infiltration and survival in the prostate. On going studies are testing the role of CTLA-4 blockade and $CD4^+$ T cell help to rescue this deletion.

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Introduction

It is well-appreciated that growing tumors suppress the anti-tumor response by at least 2 mechanisms-generalized immunosuppression and antigen-induced tolerance. The goal of this research project is to test the hypothesis that modulating costimulatory receptors expressed by T cells can reverse tolerance to prostate tumor antigens and elicit a more potent anti-tumor immune response. We use a transgenic mouse model of human prostate cancer, the **TR**ansgenic **A**denocarcinoma of the **M**ouse **P**rostate (TRAMP) model, to study T cell responses to prostatic tumors. In TRAMP mice, primary tumors develop as a consequence of prostate-specific expression of a transforming antigen, the SV40 T antigen (TAg). We use TAg as a surrogate tumor antigen. We also have two other mouse lines which bear T cell receptor transgenes that encode either MHC class I-restricted (TcR-I) or class II-restricted (TcR-II) antigen receptors. Our goal is to use these murine lines to understand how T cells develop tolerance to tumor antigens and to test whether modulation of costimulatory receptors is sufficient to overcome tolerance to tumors by understanding these basic immunologic processes.

Body

Task 1: *To determine the Developmental Stage at Which TRAMP Mice Become Tolerant to TAg:*

In the previous annual report, we described the development of a TAg-expressing vaccine to use to identify the development of tolerance in TRAMP mice. In our initial experiments, these cell-based vaccines expressed TAg and elicited a T cell response in wild-type, non-transgenic (WT) mice. However, our further studies were unable to detect a marked T cell response, despite the fact that TAg message and protein were still detected. Moreover, use of these cell lines to sensitize WT mice bearing adoptively transferred TcR-I cells (as described in Task 3, below) did not result in a proliferative response (Figure 1). These findings suggest that despite identifiable expression of TAg, these transduced cell lines are unable to elicit a potent T cell response *in vivo*. As such, we have been unable to identify a potent strategy to elicit T cell responses to TAg and could not complete this Task or Task 2. We are, however, continuing to identify ways to sensitize mice to TAg and are currently testing whether antigen-pulsed dendritic cells (as described in Task 3) may be appropriate for these two Aims.

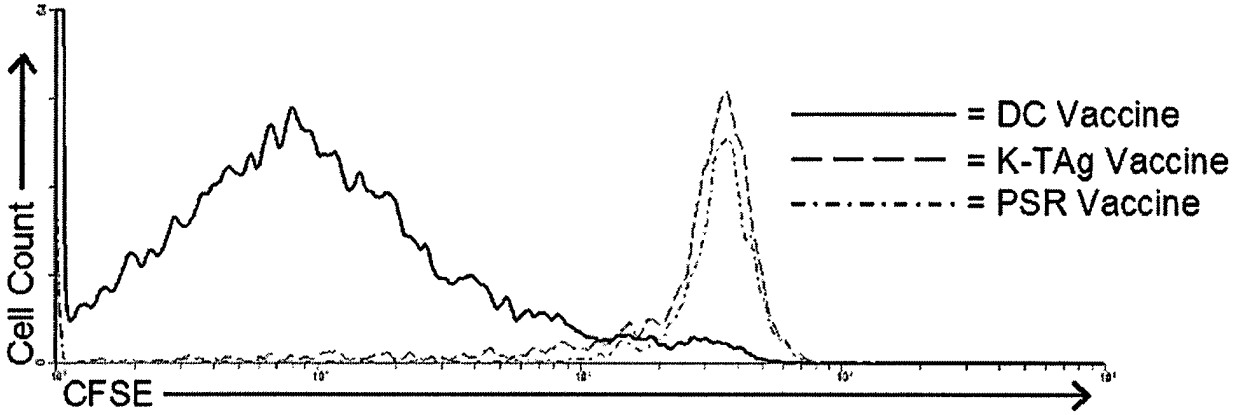

Figure 1: TAg-Expressing Cell-Based Vaccines Do Not Stimulate TAg-Specific T Cells.
TCR-I T cells were transferred via tail vain into WT/F_1 mice on day 0. Mice were vaccinated with the indicated vaccine on day 1. Vaccine DLN (DC vaccine = inguinal, K-TAg and PSR vaccine = mesenteric) were removed and analyzed on day 3 post-vaccine. CFSE histogram represents AT T cell population determined by gating on $CD8^+/K^{b(-)}$ cells.

Task 3: *To use an adoptive transfer system where transgenic T cells that recognize MHC class I- and class II-restricted TAg epitopes can be monitored to test the hypothesis that a developing prostatic tumor can tolerize naïve TAA-specific T cells.*

Many studies suggest that as a tumor develops, T cell tolerance to TAA's ensues. Most of these studies have employed transplantable tumor lines that express xenogeneic antigens that are thus highly stimulatory to the immune system. The TRAMP model presents a novel model where primary tumors develop under the developmentally regulated expression of a tissue-restricted promoter. We proposed to study T cell tolerance using the TcR-I and TcR-II transgenic lines which bear transgenes encoding TcR gene that recognize MHC class I- and class II-restricted epitopes of TAg, respectively.

The TcR-I mouse strain was bred to homozygosity on the C3H background. Lymph node cells (LNCs) from these mice were used as donor cells for transfer in TRAMP x C3H (TRAMP/F_1) mice. Similar transfers were performed using wild-type C57BL/6 x C3H (WT/F_1) as recipients or using WT C3H cells as donor cells. Donor LNC were labeled with CFSE, a fluorescent dye that distributes evenly among daughter cells as the cells divide and therefore a linear reduction of fluorescence is observed. Last year, we presented preliminary evidence that whereas WT/F_1 recipients harbor TcR-I cells without indication of proliferation, TAg expression in TRAMP/F_1 mice induces a proliferative response by TcR-I cells. Our subsequent studies have confirmed and extended those findings and are presented below.

Given the finding that TcR-I cells proliferate in TRAMP mice, we sought to determine the fate of these T cells. TRAMP/F_1 mice were transferred with TcR-I cells and the peripheral lymphoid compartments tested for the presence of TAg-specific cells over a 1 month period. In addition, we tested for TcR-I cells in the prostate, the principal site of TAg expression. Naïve T cells were labeled with CFSE prior to transfer and analyzed post-transfer by identifying TcR-I cells as $CD8^+$ and H-$2K^{b(-)}$ or by using a tetrameric reagent consisting of the TcR-I MHC restricting element and TAg peptide epitope.

Within 2 days of transfer, TcR-I cells were observed to traffic to and proliferate in the prostate-draining lymph nodes (Figure 2). At later time points (5 days post transfer), TcR-I cells were found to proliferate in the prostate-draining LNs. However, by 9 days after transfer, T cells were absent in all lymphoid tissues but still present in prostatic tissue. Somewhat surprisingly, T cells remained detectable in the prostatic tissues for up to 3 weeks (Figures3,5), albeit at relatively low levels compared to peak infiltration at 5-7 days after transfer. In WT/F_1 mice, TCR-I cells did not proliferate and did not traffic to the prostatic tissues (Figures 2,3), indicating that this response was antigen-specific. Results from these experiments indicate that expression of a tumor antigen may cause deletion of tumor-specific T cells. As such, this would be considered a somewhat novel and unexpected form of tolerance.

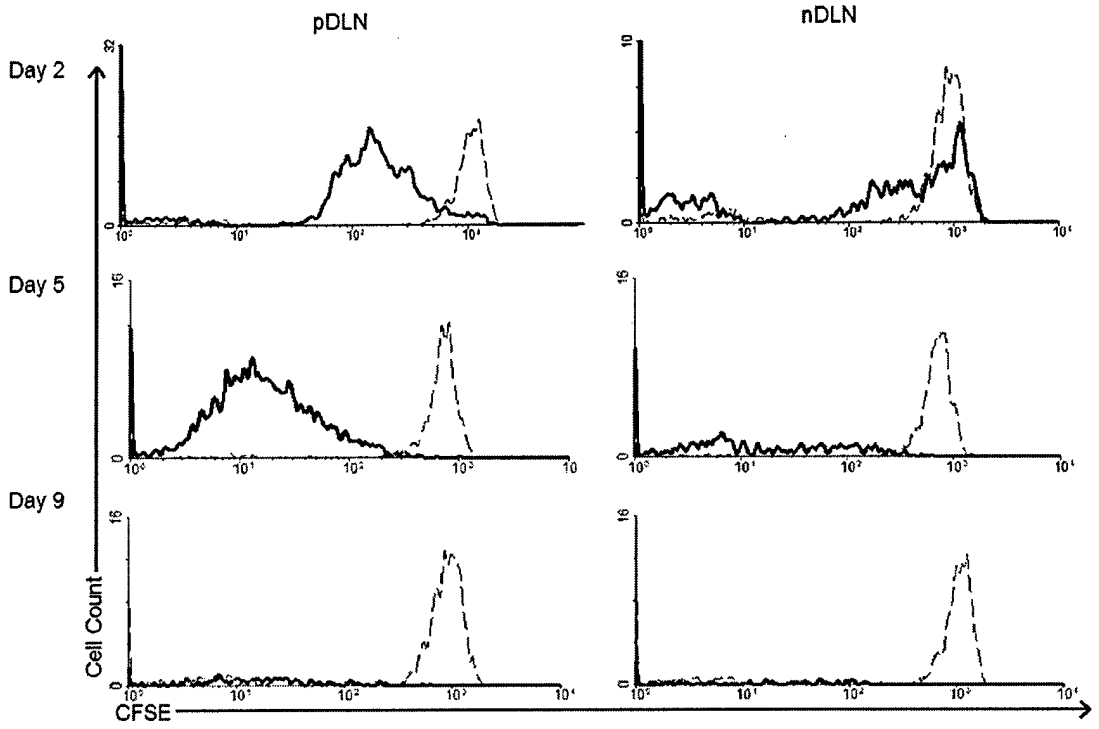

Figure 2: Ag-Specific T Cells Preferentially Traffic to and Divide in prostate-draining LNs (pDLNs) in TRAMP mice. TCR-I T cells were transferred into TRAMP/F_1 and WT/F_1 mice on day 0. pDLN (peri-aortic) and non-draining LNS (nDLN, inguinal) were removed and analyzed on the indicated days. CFSE histogram represents adoptive transfer of T cell population determined by gating on $CD8^+/K^{b(-)}$ cells. Solid line represents TRAMP/F_1 mice, dashed line represents WT/F_1 mice.

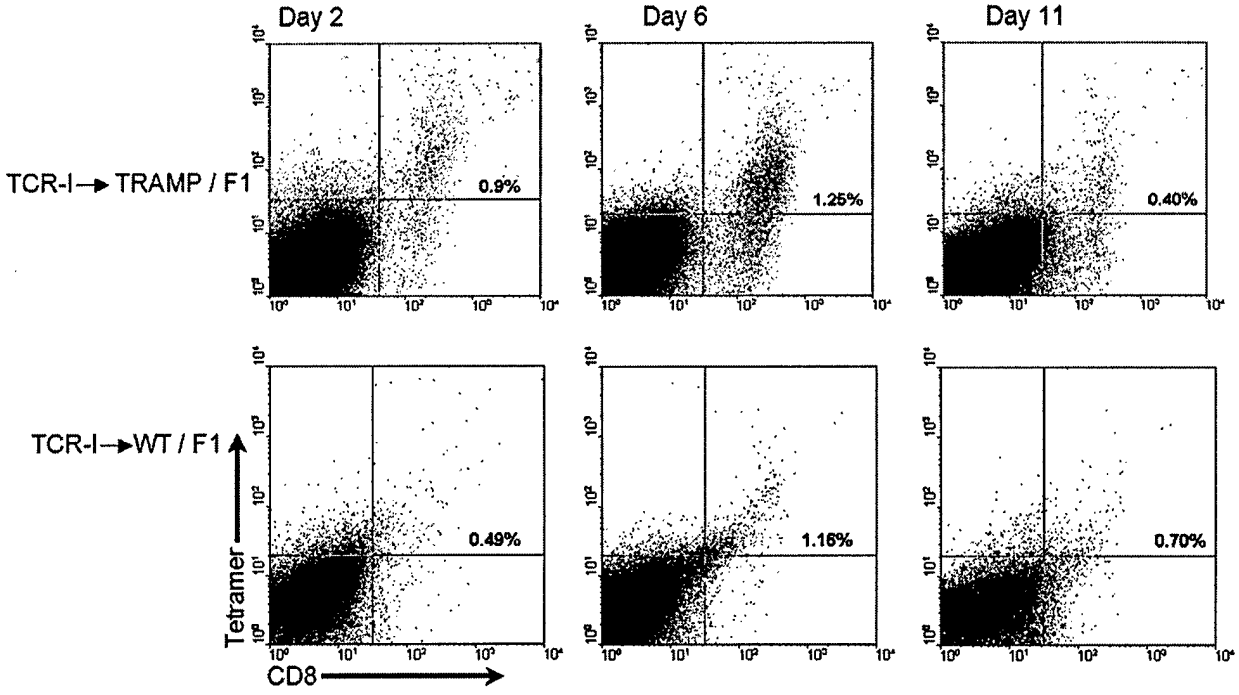

Figure 3: Ag-Specific T Cells Traffic into the Prostate in TRAMP mice.
TCR-I T cells were transferred into TRAMP/F_1 or WT/F_1 mice on day 0. Prostates were removed and analyzed on the indicated days. CD8 vs. Tetramer dotplot is the product of a lymphocyte gate applied to the FSC vs. SSC dotplot.

To identify ways to reduce deletion and promote activation of tumor-specific T cells, we sought to sensitize mice to TAg. We first attempted to use the TAg expressing vaccines originally described for Task 1. However, these vaccines did not elicit a response in WT/F_1 mice transferred with TcR-I cells (Figure 1). Therefore, we tested dendritic cells (DC) pulsed with cognate peptide antigen. DC were prepared from bone marrow in GM-CSF-supplemented cultures and pulsed with peptide overnight. The resulting DC exhibited an intermediate activation phenotype but were capable of eliciting a proliferative response by TcR-I cells transferred into WT/F_1 mice. Therefore, these antigen-pulsed DC were used to sensitized TRAMP/F_1 mice bearing TcR-I cells.

Our first observation in both TRAMP/F_1 and WT/ F_1 mice was that there was a marked increase in TcR-I cells in the vaccine-draining lymph nodes (inguinal LNs), accompanied by a dilution in CFSE fluorescence (Figure 4). This expansion was antigen-dependent as a control peptide-pulsed DC vaccine had no effect on TcR-I cells. The effects of the TAg-pulsed DC vaccine was more pronou8nced in the vaccine-draining LN than the prostate-draining LN. However, by 11 days after vaccination, deletion of TcR-I cells in all lymphoid compartments had occurred.

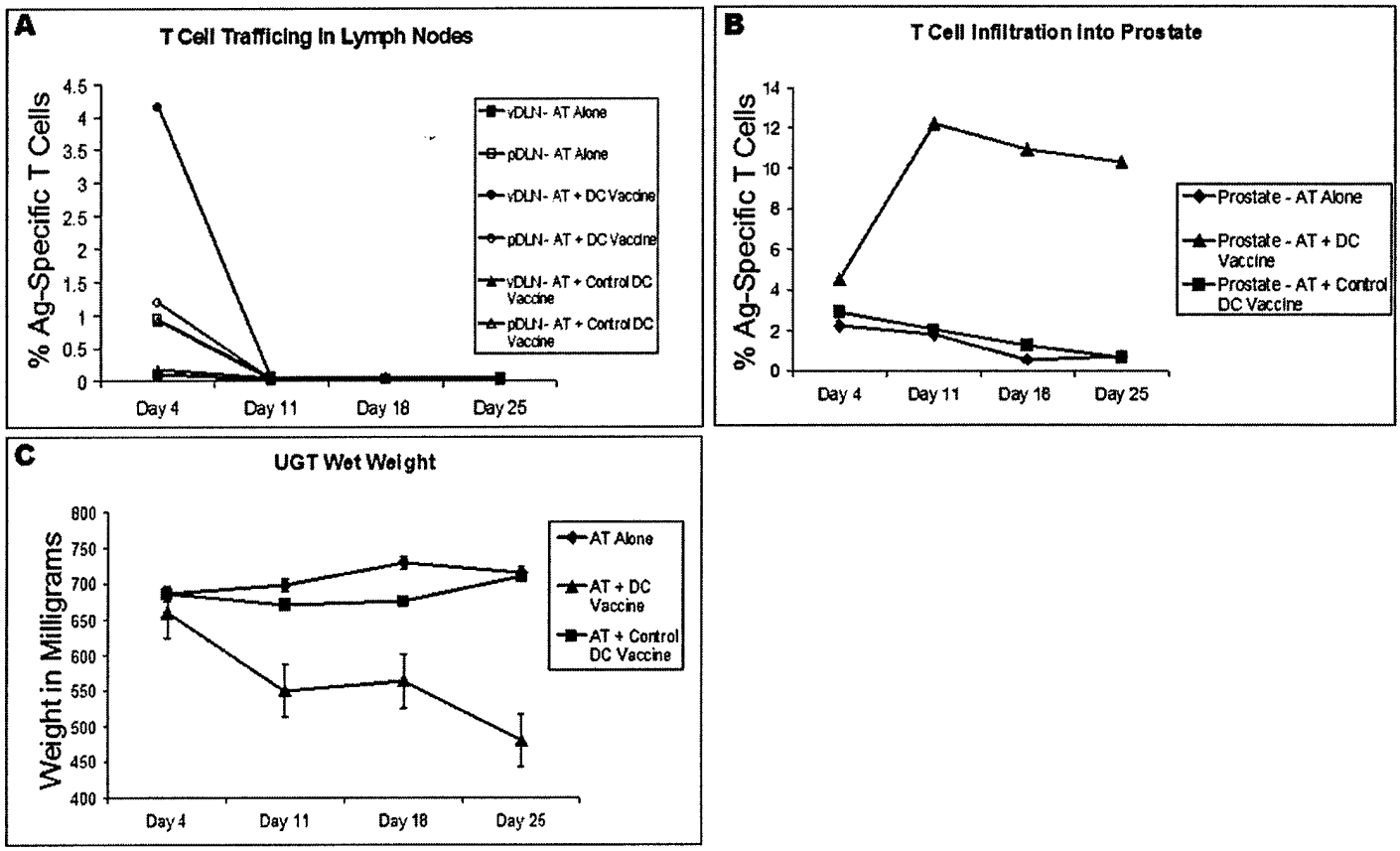

Figure 4: TAg-Pulsed DC Vaccine "Rescues" Prostate-Specific T Cells from Deletion.
TCR-I T cells were transferred into TRAMP/F_1 mice on day 0. Mice received indicated DC vaccine on day 1. pDLN, vaccine-draining LN (vDLN) and prostates were removed and analyzed on the indicated days. Percentage of Ag-specific T cells in LNs was determined by $CD8^+/Kb^{(-)}$ gate. Percentage of Ag-specific T cells in the prostate was determined by $CD8^+$/Tetramer$^+$ cells.

In the prostate, the site of endogenous antigen expression, vaccination with TAg-pulsed DC had a different effect (Figures 4,5). Although infiltration began in vaccinated mice at times similar to unvaccinated mice, T cells persisted longer in TRAMP/F_1 vaccinated mice. At day 11, when TcR-I cells had disappeared from all peripheral lymphoid organs, there were still detectable at stable numbers in the prostate tissues. Not only did the DC vaccine promote survival of T cells in the

prostate, but up to a 3-fold expansion of TcR-I cells was noted in the prostates of vaccinated TRAMP/F_1 mice. This finding suggests that after antigen-specific expansion in the vaccine-draining LNs, TcR-I cells traffic to the prostate, the site of antigen expression. TcR-I cells persisted at elevated in the prostate up to 3.5 weeks after vaccination, suggesting that a potent antigen stimulus can rescue tumor-specific T cells from deletion.

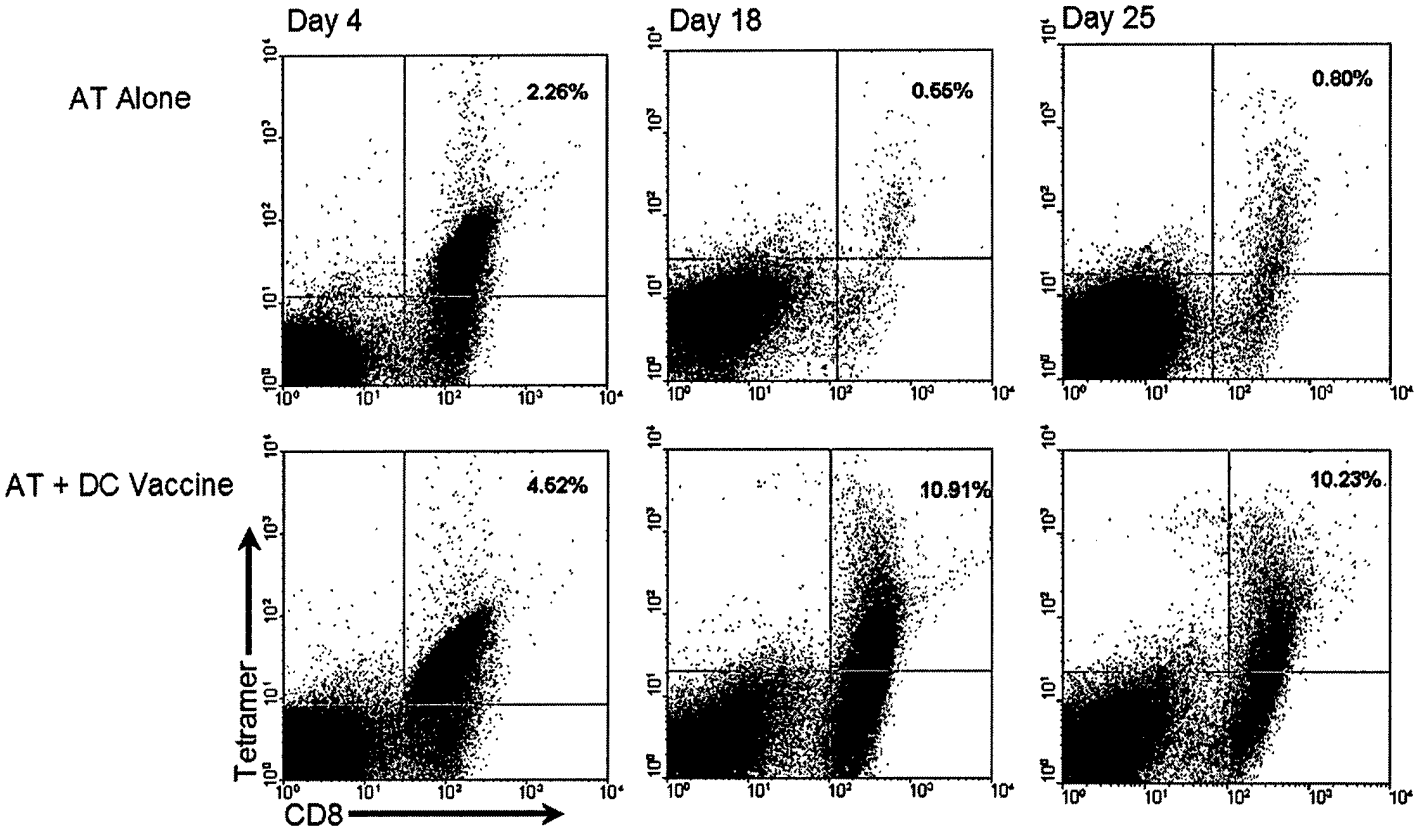

Figure 5: DC Vaccine Causes Expansion and Persistence of Prostate-Specific T Cells.
TCR-I T cells were transferred into TRAMP/F_1 mice on day 0. Mice received DC vaccine on day 1. Prostates were removed and analyzed on the indicated days. CD8 v Tetramer dotplot is a product of a lymphocyte gate applied to the FSC vs. SSC dotplot.

Interestingly, in the presence of this profound tumor-specific T cell accumulation in the prostate, preliminary results suggest that the weight of the urogenital tract in DC-vaccinated mice decreased (Figure 4). This finding suggests that the prostate-/tumor-specific T cells may induce antigen-specific destruction of the prostatic tissues in the presence of the DC vaccine. On-going histopathological studies may confirm this idea.

Key Research Accomplishments

- Establishment of adoptive transfer model
- Characterization of TcR-I cells transferred into TRAMP mice
- Identification of DC vaccine as effective in preventing deletion of TcR-I cells and promoting survival and expansion of TcR-I cells in prostate

Reportable Outcomes:

No peer-reviewed manuscripts have been published.

This work was presented by Mr. Michael Anderson, a graduate student in the laboratory, at the Keystone Symposium on Tumor Immunology, March, 2003.

Conclusions:

Our long-term goal is to understand the role of costimulatory receptors in regulating T cell tolerance to tumor antigens. Our early data suggest that TRAMP mice may exhibit both central and peripheral tolerance to TAg, a surrogate tumor antigen. We have focused our research on using the TRAMP mouse as a recipient for TAg-specific T cells.

Our findings suggest that prostate-specific T cells undergo an initial proliferative response after antigen encounter. This is followed by deletion from the peripheral lymphoid organs and the prostate, the site of antigen expression. However, sensitization with an antigen-pulsed DC vaccine rescues deletion and promotes survival of prostate–specific T cells within the prostate.

Our on-going studies are characterizing both the deletional tolerance process as well as the mechanism by which the DC vaccine rescues T cells. In addition, we are determining whether provision of prostate-specific T cell help (using a TAg-specific $CD4^+$ T cell population) can rescue T cell deletion. Finally, we are continuing to pursue studies that characterize T cell tolerance in TRAMP mice by developing novel vaccination approaches to elicit anti-TAg T cell responses.

References:

none